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THE UNIVERSITY OF ALBERTA

A NUCLEAR MAGNETIC RESONANCE STUDY OF THE MANGANESE(II) -COENZYME A COMPLEX IN SOLUTION

BY

(C) YVON THERIAULT

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS

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OF

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DEPARTMENT OF CHEMISTRY

EDMONTON , ALBERTA

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled

"A NUCLEAR MAGNETIC RESONANCE STUDY OF THE MANGANESE(II)-COENZYME A COMPLEX IN SOLUTION "

submitted by YVON THERIAULT in partial fulfilment of the requirements for the degree of Master of Science.

George Kotowych
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Nov 21 1978
Date

ABSTRACT

The longitudinal and transverse relaxation rates of the three phosphorus and of the four of the proton resonances of coenzyme A(CoA) were measured in solutions of free CoA and of the CoA-Mn(II) complex. Experiments were carried out in H_2O and D_2O ,at different values of the pH and at 287°K and 298°K.

The value of the dipolar correlation time τ_c for the CoA-Mn(II) complex that is needed in the distance calculations was obtained from water proton relaxation data at 60 and 100 MHz for a solution where most of the Mn(II) ions are complexed. The number of water ligands bonded to the Mn(II) in the complex is assumed to be either two or three for the 0.062 M CoA solution.

By observing the paramagnetic effects of the weakly bound metal ions on the longitudinal relaxation rates of the three phosphorus nuclei as determined by the Fourier transform method, the distances from the bound metal to the three phosphorus nuclei were calculated. For two water molecules ($\tau_c = 6.6 \times 10^{-10}$ sec), these distances are (3.8 - 4.0)Å, (3.9 - 4.0)Å, and (3.8 - 4.0)Å to the 3'-, β- and α-phosphorus nuclei, respectively. For three water molecules ($\tau_c = 4.0 \times 10^{-10}$sec), the corresponding three distances are (3.5 - 3.7)Å, (3.6 - 3.7)Å and (3.5 - 3.7)Å respectively. These metal to phosphorus distances indicate a predominantly symmetrical inner sphere complex,establishing the direct manganese(II) coordination by the oxygens of the phosphate groups and yielding further information about the nature of the weak one to one complex between CoA and manganese (II) ions.

Proton longitudinal relaxation rates at 60 and 100 MHz were also employed to calculate distances between the bound Mn(II) ions and the four selected protons AH(8), AH(2), AH(1') and PH(3'). For two water molecules ($\tau_c = 6.6 \times 10^{-10}$ sec), the four distances are (4.7 - 4.8)Å, (6.1 - 6.5)Å, (5.6 - 6.4)Å and 6.0 Å, respectively. For three water molecules ($\tau_c = 4.0 \times 10^{-10}$ sec), the four distances are (4.4 - 4.5)Å, (5.6 - 6.1)Å, (5.2 - 5.9)Å and (5.6 - 5.7)Å, respectively.

These Mn(II) to proton distances together with the phosphorus results were used to construct models for the conformation of the CoA-Mn(II) complex in solution.

For experiments carried out on 0.010 M CoA, a correlation time previously determined for propionyl CoA ($\tau_c = 2.0 \times 10^{-10}$ sec) was used for the distance calculations. Within experimental error, the distances remain unchanged. The distances from the bound metal to the 3'-ribose, β- and α-phosphorus nuclei are 3.5 Å, 3.6 Å and 3.7 Å, respectively. The corresponding distances for the three selected proton nuclei, AH(8), AH(2) and AH(1') are 4.6 Å, 6.5 Å and 5.9 Å, respectively.

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SYMBOLS

GREEK SYMBOLS

β = Bohr magneton, 0.927×10^{-20} erg G^{-1}

γ_I = magnetogyric ratio for a nucleus of spin I

η = viscosity of solvent in poise (cgs)

μ = micro

$\Delta\nu$ = line width (Hz) of a resonance line at half height

τ = delay time

τ_c = dipolar correlation time

τ_e = hyperfine (scalar) correlation time

τ_m = chemical exchange lifetime

τ_R = rotational correlation time

ω_I = nuclear Larmor frequency in rad sec^{-1}

ω_s = electron Larmor frequency in rad sec^{-1}

ROMAN SYMBOLS

$A/\hbar$ = hyperfine coupling constant in rad sec^{-1}

a = effective radius of the complex

E_R = activation energy for the rotation of the complex

h = Planck's constant, 6.63×10^{-27} erg sec

I = nuclear spin

Kd = dissociation constant of the complex

k_f = forward rate constant

k = Boltzmann's constant, 1.38×10^{-16} erg deg^{-1}

M = molar

mm = millimeter

Mn_b = bound Mn(II) concentration

P_m = fraction of metal ion bound ligands

q = number of ligand nuclei bound to the metal ion in the complex

r = distance between nucleus and paramagnetic centre

R = ideal gas constant

T = temperature in degrees Kelvin

T_1 = longitudinal relaxation time

T_2 = transverse relaxation time

T_{1e} = electron longitudinal relaxation time

T_{2e} = electron transverse relaxation time

$1/T_1(0)$ = longitudinal relaxation rate in the absence of Mn(II) ions

$1/T_2(0)$ = transverse relaxation rate in the absence of Mn(II) ions

$1/T_1(Mn)$ = longitudinal relaxation rate in the presence of Mn(II) ions

$1/T_2(Mn)$ = transverse relaxation rate in the presence of Mn(II) ions

$1/T_{1p}$ = paramagnetic contribution to the longitudinal relaxation rate

$1/T_{1M}$ = longitudinal relaxation rate of a nucleus bound near a paramagnetic centre

CHEMICAL ABBREVIATIONS

ATP = adenosine 5'-triphosphate

CoA = coenzyme A

DTT = dithiothreitol

EDTA = ethylenediamine tetraacetic acid

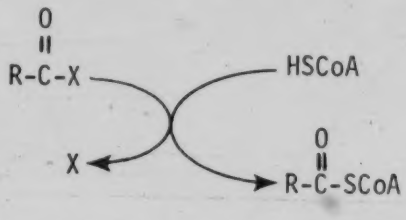
NADP = nicotinamide adenine dinucleotide phosphate

Tris = tri-hydroxymethyl-aminomethane

CHAPTER ONE

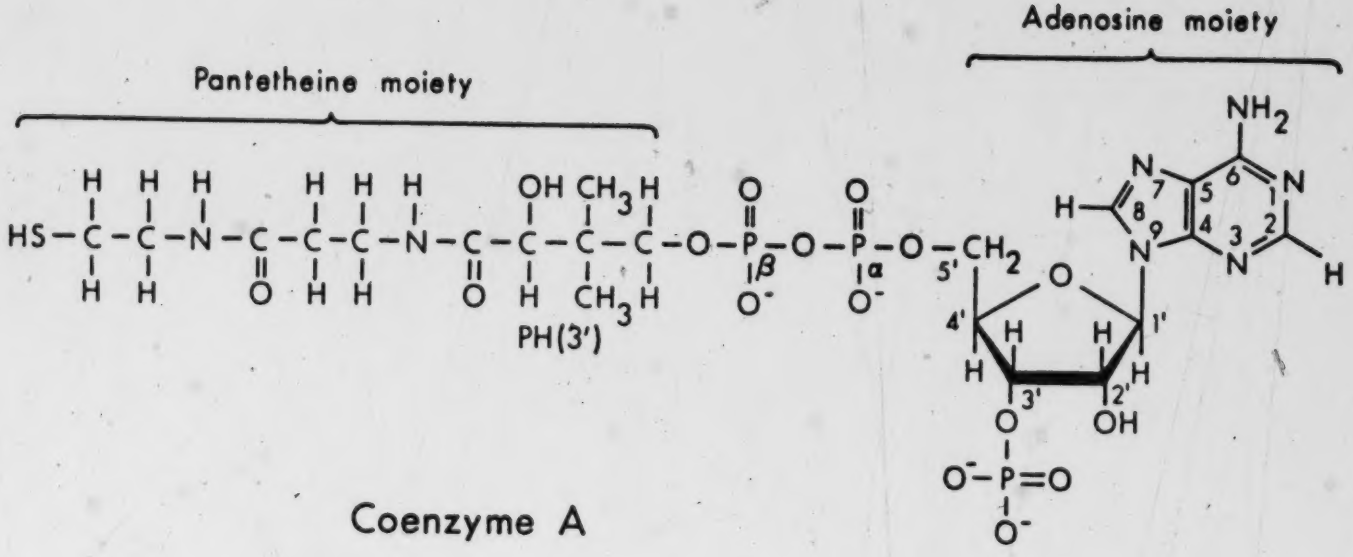
INTRODUCTION

Coenzyme A (CoA) was first studied by Lipman in 1945 and its structure (Figure 1) was elucidated a few years later (1). As shown in the scheme below, CoA acts as an acyl carrier and as such plays a vital role in metabolic pathways (1).



CoA is present in humans, animals, plants and in micro-organisms. However, its biosynthesis in higher organisms depends on an external supply of pantothenic acid since these organisms are unable to synthesize this vitamin. It participates widely in a great number of reactions involved in various metabolic pathways. Since its first successful total synthesis by Khorana et al. (2), studies of CoA-related reactions have been intensified in an effort to elucidate the structural involvement of the pantothenic acid and adenine moieties in its various biological activities. Many scientists have been interested in the underlying chemical mechanisms and have synthesized various structural analogues to analyse their association with a variety of enzymes by means of competition studies and by the investigation of the effects of these analogues on bacterial growth. The number of enzymes requiring CoA or its derivatives is at least 63 of which 17 also require Mg(II) ions for their activity (1). The obligatory requirement of these various enzymes for the divalent cation might also be

FIGURE 1. The structure of CoA.



Coenzyme A

fulfilled by Mn(II) ions since the chemistry of these two metal ions is very much alike. However, unlike Mg(II), Mn(II) has spectroscopic properties which make it possible to probe the nature of the enzyme-Mn(II) interactions. Ideally, the function of these enzymes in the presence of the Mn(II) probe is usually checked for comparable activity and kinetic properties at the active site to insure that the enzymatic activity is not disturbed to any critical degree from that observed for Mn(II) ion.

The conformational change at the reaction centre of an enzyme may involve the specific binding of the metal ion to the enzyme surface via a Mn(II) nucleotide substrate to form an active ternary complex. The observation of enzyme binding sites for the Mn(II)-ATP complex and various competition studies involving metal ion-nucleotide complexes suggest that the actual reacting species in the catalytic mechanism may well be the binary Mn(II)-nucleotide complex. In this case, the geometry of the molecule in the binary complex can be constructed and compared with its conformation in the ternary enzyme complex. The shapes of large free molecules in solution are usually different from those of enzyme-bound molecules. This thesis will consider a possible model for the favored conformation of the CoA-Mn(II) complex in solution as measured by phosphorus and proton magnetic resonance studies.

Previous studies on the conformation of CoA were carried out by measuring various parameters such as NMR shifts, coupling constants and relaxation times which established the geometric arrangement and conformation of various portions of free CoA or bound CoA. For example,

Mieyal et al. (3) in their NMR study of benzoyl CoA molecules in solution have shown that a specific intramolecular complex, in which the two rings are stacked, exists for approximately 65 % of the molecules, at a temperature of 308° K and at a pD of 6.5 . Wilson et al. (4) have reported strong evidence using coupling constants derived from CoA and pantetheine for a conformation involving hydrogen bonding between the hydrogen atom of the β-alanyl NH group and possibly either the pyrophosphate oxygen atom or the 2'-OH of the sugar moiety. Further evidence that an intramolecular interaction exists between the phosphopantetheine side chain and the ribose phosphate and also the adenine residue was provided by Lee and Sarma (5). They found that in aqueous solutions, a maximum of 30 % of the total population of acetyl CoA and CoA are present in several possible folded conformations which are in rapid equilibrium with simple linear conformers. One such model, based on observed shifts, has the pantetheine side chain folded over the purine base. Fung et al. (6) have analysed the diamagnetic and paramagnetic effects of the enzyme bound propionyl CoA and determined the positions of both propionyl CoA and pyruvate substrates on trans-carboxylase. The spatial arrangement of bound propionyl CoA that was obtained was U shaped about the Co(II) centre. Buttlaire, Cohn and Bridger(7) observed a weak, one to one complex between Mn(II) and CoA with a dissociation constant, Kd, of 1.1×10^{-3} M at pH 8.0 and 298°K. The dissociation constant was determined using ESR techniques from a titration of 4.0×10^{-3}M CoA with Mn(II) ions. However, the effect of the Mn(II) ions on the relaxation rates of the three ^{31}P nuclei was not

analysed so that the exact character of the complex is not known.

In the present study, longitudinal relaxation time experiments have been carried out in order to calculate distances from the bound Mn(II) to the three phosphorus and to the four proton nuclei. From these physical parameters, it can be proposed that the nature of the CoA-Mn(II) complex and its structure can be compared with the conformation of free CoA and with the ATP-Mn(II) complex. Recently, Fazakerley et al. (8) has shown through the use of nuclear magnetic relaxation and of dipolar shifts by lanthanide ions that the dephospho CoA complex exists in an ^2E conformation with the metal coordinated to four oxygen donors of the pyrophosphate moiety. The adenine base is oriented in the anti conformation. The pyrophosphate portion is folded backward while the remainder of the chain is extended downward.

CHAPTER TWO

THEORY

Mapping of the Mn(II) ligand complex can be carried out by analysing the effect of the metal ion on the relaxation rates of nuclei in the complex under certain conditions. In a dilute solution, the nuclear relaxation rate is mainly dominated by the strong pair-wise interaction between the spin of an unpaired electron and the nucleus.

The contribution of the Mn(II) ion to the relaxation rate, $1/T_{ip}$, is obtained by observing the relaxation rate in the presence of Mn(II), $1/T_i(Mn)$, and subtracting from this value the the relaxation rate in the absence of the paramagnetic ion, $1/T_i(0)$, (9, 10) :

$$1/T_{ip} = 1/T_i(Mn) - 1/T_i(0) \qquad [1]$$

where i = 1 or 2. $1/T_1$ is the longitudinal relaxation rate and $1/T_2$ is the transverse relaxation rate.

The effect of the complexed Mn(II) ions in various environments ,i.e. free aquo-ions and metal ions bound to the CoA in a binary complex, on the longitudinal relaxation rates of water protons is given by equation [2] (11) :

$$1/T_{1p} = \frac{Mn(H_2O)}{Mn_t T_{1p}(H_2O)} + \frac{Mn(CoA)}{Mn_t T_{1p}(CoA)} \qquad [2]$$

where $Mn_t = Mn(H_2O) + Mn(CoA)$ is the total manganese concentration,

and $Mn(H_2O)$ is the molar ion concentration present in the aquo-complex and Mn(CoA) is the concentration of bound Mn(II) in the CoA binary complex. Using the dissociation constant, Kd, of 1.1×10^{-3} M at 298°K obtained at a CoA concentration of 4 mM (7), and assuming that this Kd is also applicable at 287°K, we have estimated that the free metal ion concentration was less than 2 % ($Mn(H_2O)/Mn_t$) under our experimental conditions. Consequently, we neglect the first term in equation [2] and $Mn(CoA)/Mn_t$ is approximately equal to one.

The relationship between the observed relaxation rate due to the paramagnetic ion $1/T_{ip}$ and the inner sphere relaxation time T_{iM} is given by Luz and Meiboom (9) and by Swift and Connick (10) as:

$$1/T_{1p} = \frac{P_m q}{T_{1M} + \tau_m} + P_m q T_{os}^{-1} \qquad [3]$$

$$1/T_{2p} = \frac{P_m q}{\tau_m} \left[\frac{T_{2M}^{-1} (T_{2M}^{-1} + \tau_m^{-1}) + \Delta\omega_m^2}{(T_{2M}^{-1} + \tau_m^{-1})^2 + \Delta\omega_m^2} \right] + P_m q T_{os}^{-1} \qquad [4]$$

where P_m = [metal ion] / [observed ligand], q is the number of water ligands(q = 2 or 3) or CoA ligands (q = 1) bound to the Mn(II) ion, τ_m is the exchange lifetime of the ligand in the complex and $\Delta\omega_m$ is the chemical shift between the bound and the bulk sites. T_{os}^{-1} is the outer sphere term due to the dipole-dipole interaction between the paramagnetic ion and the CoA nuclei in the bulk solution. This term

is proportional to the number of spins per ml of solution. For an aqueous solution of 100 μM Mn(II) at 20 °C, the normalized outer sphere relaxation term is 1.1×10^5 sec^{-1}, and is responsible for 6% of the observed water proton relaxation (12). If it is assumed that only the free metal ions in solution can contribute to the outer sphere effect in the CoA system, since the closest approach between a CoA ligand in the bulk solution and the CoA-Mn(II) complex is larger than the closest approach between the free aquo-complex and the CoA ligand and further assume that the above calculated outer sphere value is also applicable to this system, then an estimation for the outer sphere term is possible for the water proton relaxation data in Table I. That is, for 2% free Mn(II) ions in solution, the outer sphere effect contribution to the observed water relaxation is approximately 1%. The outer sphere effect contribution to the relaxation rates of the various nuclei of CoA is also probably negligible since a comparison of solutions that contain different concentrations of Mn(II) ions shows that the slope of the plot of $[\text{ CoA }]\left[\frac{1}{T_1(\text{Mn})} - \frac{1}{T_1(0)}\right]$ versus [Mn(II)] (Figure 5) is a simple function of the [Mn(II)]. This contribution is also assumed to be negligible for the solutions containing 10% free Mn(II) ions (samples 7 and 12 in Tables III and VII respectively).

When fast-exchange conditions apply, i.e. $T_{1M}^{-1} \ll \tau_m^{-1}$ and $(T_{2M}\,\tau_m)^{-1} \gg T_{2M}^{-2}$, $\Delta\omega_m^{\,2}$, the above equations become (9, 13, 14):

$$1/T_{ip} = P_m\, q/\, T_{iM} \qquad [5]$$

The relaxation rates of a nucleus bound near a paramagnetic centre are given by the Solomon-Bloembergen equations (15,16, 17,18):

$$T_{1M}^{-1} = \frac{2}{15} \frac{S(S+1)\, g^2 \beta^2 \gamma_I^2}{r^6} \left[\frac{3\tau_{c1}}{1+\omega_1^2 \tau_{c1}^2} + \frac{7\tau_{c2}}{1+\omega_s^2 \tau_{c2}^2} \right] + \frac{2}{3} S(S+1) \frac{A^2}{\hbar^2} \left[\frac{\tau_{e2}}{1+\omega_s^2 \tau_{e2}^2} \right] \qquad [6]$$

$$T_{2M}^{-1} = \frac{1}{15} \frac{S(S+1)\, g^2 \beta^2 \gamma_I^2}{r^6} \left[\frac{7\tau_{c1}}{1+\omega_1^2 \tau_{c1}^2} + \frac{13\tau_{c2}}{1+\omega_s^2 \tau_{c2}^2} \right] + \frac{1}{3} S(S+1) \frac{A^2}{\hbar^2} \left[\tau_{ei} + \frac{\tau_{e2}}{1+\omega_s^2 \tau_{e2}^2} \right] \qquad [7]$$

where γ_I = magnetogyric ratio, 2.68×10^{14} rad sec^{-1} $gauss^{-1}$ for ^1H,

r = nucleus to metal distance in Å,

ω_s = angular electron precession frequency in rad sec^{-1},

ω_I = angular nuclear precession frequency in rad sec^{-1},

$A/\hbar$ = scalar coupling constant in rad sec^{-1},

β = Bohr magneton, 8.80×10^6 rad sec^{-1} $gauss^{-1}$,

g = Lande g factor, 2.00 for Mn(II),

S = total electron spin, 5/2 for Mn(II),

τ_c = correlation time (sec) which characterizes the frequency

or variation of the Mn(II)-nuclear interaction caused by molecular motion.

The correlation times in the above equations are defined as:

$$1/\tau_{ci} = 1/\tau_R + 1/T_{ie} \qquad [8]$$

$$1/\tau_{ei} = 1/\tau_m + 1/T_{ie} \qquad [9]$$

where τ_R = rotational correlation time of the CoA molecule, which is also equal to that for the whole complex when isotropic rotation is present,

τ_m = lifetime of the observed nucleus in the complex,

$1/T_{1e}$ = longitudinal electron spin relaxation rate,

$1/T_{2e}$ = transverse electron spin relaxation rate.

In the present analysis, the difference between τ_{c1} and τ_{c2} is ignored since for small Mn(II) binary complexes, $T_{1e} = T_{2e}$ (12,14).

It is important to note that under the present experimental conditions $(\omega_s \tau_c)^2 >> 1$ and $(\omega_s \tau_{e2})^2 >> 1$ so that equations [6] and [7] reduce to :

$$1/T_{1M} = (C/r)^6 \left[\frac{3\tau_c}{1+\omega_I^2 \tau_c^2} \right] \qquad [10]$$

$$1/T_{2M} = \frac{1}{2}(C/r)^6 \left[\frac{7\tau_c}{1+\omega_I^2 \tau_c^2} \right] + \frac{1}{3} S(S+1)\left(\frac{A}{\hbar}\right)^2 \tau_{e1} \qquad [11]$$

where C is the product of the above physical constants and equal to 601 and 812 for Mn(II) ion interactions with phosphorus and protons respectively (14).

The temperature dependence of τ_R is given by the exponential function:

$$\tau_R = \tau_R^{\circ} \times \exp(E_R/RT) \qquad [12]$$

where τ_R° = a constant,

E_R = the activation energy for rotational motion,

R = the ideal gas constant and

T = the temperature in degrees Kelvin.

It is possible to estimate τ_R by the use of the Stokes-Einstein equation :

$$\tau_R = \frac{4\pi\eta a^3}{3kT} \qquad [13]$$

where η = the viscosity,

a = the effective radius of the molecule and

k = the Boltzmann's constant.

CHAPTER THREE

EXPERIMENTAL SECTION

Materials

All glassware necessary to handle the purified CoA was washed initially with soap and water. The glassware was then immersed consecutively in the following solutions for at least three hours to remove all traces of metal impurity : 20% HNO_3, 0.1% NH_4OH and finally in a 0.05M EDTA solution at pH 7.2. An ample supply of distilled water was used to rinse the glassware between the soakings.

The reagents used were Chelex-100 (Bio-Rad), Tris (Schwartz-Mann), KCl (Fisher), dithiothreitol (Sigma), $MnCl_2.4H_2O$ (Fisher), Mn(II) Reference Standard Solution, 1000 ppm (Fisher), Yeast CoA (Sigma), CoA oxidized (Sigma) and CoA (P.L. Biochemicals). The concentration of one Mn(II) stock solution (4.05×10^{-5} M) was determined by ESR. This was done by matching two standard Mn(II) solutions prepared from the Fisher certified Manganese Reference Standard Solution with the unkown Mn(II) sample. The second stock Mn(II) solution (2.00×10^{-3}M) was calculated on the basis of weight.

Trace metal contaminants were removed from all buffers by elution through a Chelex-100 column with a 0.5 cm^2 x 28 cm resin bed. D_2O was distilled once and the water was triply distilled. The triply distilled water had a T_1 of 3.5 sec at 300 °K, pH 5.3. Quoted pH values are uncorrected pH meter readings. For D_2O solutions, pD may be obtained by adding 0.4 to the uncorrected pH values (19).

Column Preparation

Chelex-100 was treated with 1N HCl, 1N NaOH and methanol and rinsed with doubly distilled water after the acid and base treatment. Then the resin in the Na^+ form was equilibrated with either 0.05 M Tris/ 0.1M KCl or 0.09 M Tris/ 0.1M KCl, pH 7 .Chelex-100 was chosen over Dowex 50W-X8 because a smaller column could be used. Its efficiency was checked by eluting a sample of ATP and measuring the proton T_1 values, which agreed with previous results using a longer Dowex column (20). CoA powder was stored at -20 °C in a desiccator and was used usually within 1 month after arrival.

CoA was washed free of metal contaminants by passage through the Chelex-100 column. Prior to the elution of CoA (0.03M, pH 6.5-7.5) from the column ,the bottom of which was fitted with a small glasswool plug, two void volumes of triply distilled water which had been boiled for 15 min and cooled in a nitrogen atmosphere were added to make the resin low in ionic strength and in oxygen. Dextran 2000 was used in the calculation of the void volume. The CoA sample was eluted under a nitrogen atmosphere in a glove bag with the degassed, triply distilled water at a flow rate of one milliliter per minute. Roughly 20 ml of solution were collected at room temperature and were then lyophilized. NMR experiments on the samples were usually carried out within 10 hours. The samples were stored at 4 °C if not used immediately. The resin was not regenerated.

Sample Preparation

For the NMR experiments, the eluted solution was lyophilized.

In some of the earlier experiments, the powder was then dissolved in D_2O and lyophilized again to remove any H_2O remaining in the powder. However, on two occasions, the samples turned pink upon freeze-drying a second time so that in later experiments, it was lyophilized only from the eluent. The residue was diluted with 1-2 ml of the buffer ($[CoA] \simeq 10^{-1}$ M). A 10-20 microliter aliquot of this solution was diluted with doubly distilled water in a 25.00 ml volumetric flask ($[CoA] \simeq 10^{-4}$ M) and its concentration measured spectrophotometrically at ambient temperature at 260 nanometers on a Gilford modified Beckman DU Model spectrophotometer using an extinction coefficient of 1.68×10^4 cm^{-1} M^{-1} (21) and a 1-cm cell. Samples of 300-350 microliters were prepared in 5 mm NMR tubes (Wilmad) and were degassed five times using the freeze-pump-thaw technique on a vacuum line before sealing. Nitrogen gas was also occasionally used for deaeration.

In order to minimize the possibility of paramagnetic impurities influencing the line width and the T_1 experiments, the pH adjustments of the various solutions were made on the resin which was previously equilibrated with a buffer at the proper pH value.

The various concentrations and pH readings for the solutions are given in the Tables together with the summary of the T_1 data. At pH 8.0, the 3'-phosphate moiety and the pyrophosphate linkage are doubly ionized whereas at pH 5.0, the former is a mono-anion and the latter is still doubly charged (5). The ^1H NMR chemical shifts of CoA and its derivatives at neutral and alkaline pH have the same values (3).

Proton NMR Measurements

The CoA proton NMR spectra were taken either on a Varian HA-100-15 (100 MHz) using the Digilab FTS/NMR-3 FT, the pulse unit FTS/NMR 400-2 and the NOVA 1200 computer or on a Bruker WP-60 (60 MHz) using the BNC-12 computer.

Samples were prepared with a constant CoA concentration (0.062 M) and a Mn(II) ion concentration which varied from 1.7×10^{-6} M to 18×10^{-6} M. The amount of free Mn(II) in these solutions was calculated to be less than 2% of the total Mn(II) concentration, based on a dissociation constant for the binary complex, Kd, of 1.1×10^{-3} M at 298°K (7). In some of the measurements of the proton T_1 value at 100 MHz by the inversion recovery technique, the strong HDO resonance was reduced by continuous wave saturation using a Hewlett Packard 5100 B frequency synthesizer.

Water proton NMR relaxation times (T_1) were measured using the Varian HA-100-15 NMR and the Bruker WP-60 NMR spectrometers. The samples, 40 microliters, were placed inside an external reference capillary tube and inserted into a 5 mm tube containing deuterated acetone for the lock signal. The free metal concentration of the prepared samples at 0.062 M was less than 2%. But the free metal in the 0.010 M samples was nearer 10%.

The sulfhydryl group of the CoA is susceptible to oxidation, forming the CoA disulfide dimer. The average amount of CoA that oxidized was 12% as calculated from:

$$\% \text{ oxidation} = \frac{\text{peak height of the dimer resonance} \times 100}{\text{dimer peak height} + \text{monomer peak height}} \quad [14]$$

The AH(2) resonance for the oxidized compound appears 3 Hz upfield from the AH(2) resonance for the reduced compound. The upfield signal was ascribed to the CoA dimer AH(2) peak as follows. A mixture of CoA dimer and monomer was reduced in an electrolysis cell and a comparison of the ^1H spectra of the solution before and after the reduction shows that the upfield peak completely disappears. Addition of CoA dimer to CoA increases this upfield resonance line.

The temperature regulation in the above NMR instruments was carried out using the Bruker B-ST 100/700 temperature control unit. The basic pulse sequence used for the measurement of T_1 values was $(180°\text{-}\tau\text{-}90°\text{-collect-}5T_1)_n$.

$1/T_{2p}$ was calculated from the line widths at half height, $\Delta\nu_{\frac{1}{2}}$(Hz) as follows : $1/T_{2p} = \pi\Delta\nu_{\frac{1}{2}}(Mn) - \pi\Delta\nu_{\frac{1}{2}}(0)$ [15]

where $\Delta\nu_{\frac{1}{2}}(Mn)$ is the line width in the presence of Mn(II) and $\Delta\nu_{\frac{1}{2}}(0)$ is the line width in the absence of Mn(II).

^{31}P NMR Measurements

^{31}P NMR spectra were obtained on a Bruker HFX-90 NMR spectrometer (36.4 MHz for ^{31}P) interfaced with a Nicolet 1085 computer. The proton decoupled FT spectra were recorded with a sweep width of 2000 Hz and 8 K data points . The pulse sequence used was as mentioned above.

Samples in a 5 mm NMR tube were prepared either in H_2O or D_2O. Data were obtained at two temperatures, 287 and 298 degrees Kelvin.

The temperature regulation was carried out as mentioned above.

For D_2O solutions, the instrument was locked to the solvent ^2H resonance. For H_2O solutions, the 5 mm NMR tube was inserted inside a 10 mm tube, and D_2O was placed in the outer tube. Sixty to eighty pulses were needed in the inversion recovery sequence to obtain a good signal to noise ratio. Spectra were obtained at eight τ values in the inversion recovery sequence per sample. To obtain one set of T_{1p} data, the experiments lasted 6-9 hours.

The oxidation of the sulfhydryl group is not noticeable in the ^{31}P spectra. ^{31}P spectra of both the oxidized and reduced forms of CoA are identical.

One pair of samples with a CoA concentration of 0.010M in D_2O was also run in a 10 mm tube at 287 °K.

Treatment of the Data

The range of τ values employed in the measurement of the T_1 values for the various solutions did not exceed $2T_1$. The T_1 values were calculated from the peak heights of resonances with line broadening; the line broadening was introduced to increase the accuracy of the measurements.

The height of the resonance signal is proportional to the magnitude of the magnetization. According to the Bloch equations, T_1 is related to the magnitude of the magnetization as given by equation [16] (22):

$$\ln(1-A/A_\infty) = -\tau/ T_1 + \ln 2 \qquad [16]$$

where A is equal to the amplitude of the resonance signal following the 90° pulse and A_∞ is the height of the signal for a very long delay interval between the 180° and the 90° pulses. A_∞ is also the equilibrium value. The experimental data were analyzed by a computer least-squares fit to a linear function to yield T_1 with an uncertainty of ±10%.

A rough estimation for T_1 is found from the τ value at at the null point. Under this condition, A = 0 and the above equation becomes

$$T_1 = \tau / \ln 2 \qquad [17]$$

CHAPTER FOUR

RESULTS AND DISCUSSION

Experimental Errors

The calculation of the T_{1M} value for a given experiment involves the subtraction of two relaxation rates, each with an uncertainty of ±10%. The average relative standard deviations in the T_{1M} obtained from six experiments are 42% for the phosphorus and 16% for the proton nuclei obtained from four experiments (Tables IIA and V). Sloan and Mildvan (23), in a similar study, obtained 19% and 26% as the calculated uncertainties in the longitudinal relaxation data for these nuclei. The uncertainty in the individual values due to the experimental method, the variation in the mode of preparing CoA solutions and as well as in the source of CoA are the principal causes of these large average relative standard deviations in the T_{1M} values. Further uncertainties in the distances calculated from the T_{1M} values come from errors due to the theoretical and experimental uncertainty in the calculation of the correlation time from water relaxation rates :i.e. τ_c was calculated to be 6.6×10^{-10} or 4.0×10^{-10} sec, assuming either two or three water ligands in the CoA-Mn(II) complex,(q = 2 or 3), respectively ,and a Mn(II) to proton distance in the complex of 2.87 Å. The overall uncertainty in the calculated distances is then ±10% (24). The average standard deviation in $P_m T_{2p}^{-1}$ is 69% and 58% for the phosphorus and proton nuclei respectively (Tables IIB and V). Sloan *et al.* (23) reported an uncertainty of 37% for the phosphorus transverse relaxation rates as determined from the line width at half height. Their corresponding proton transverse relaxation rates were calculated using

pulse methods and therefore no direct comparison is made with the ^{1}H $P_m\ T_{2p}^{-1}$ values of CoA.

Stability of CoA

The stability of CoA was followed by monitoring the AH(2) resonance for the oxidized compound (equation [14]). The rate of CoA disappearance (originally 100 % monomer) was obtained by recording proton NMR spectra for a CoA solution over a five day period. The experimental conditions were : [CoA] = 0.042 M from P.L. Biochemicals in 0.030 M Tris containing 0.070 M KCl solution, pH 9.9 at a temperature of 29± 2°C. EDTA was 0.0005 M. A volume of 500 microliters was transferred to a 5 mm NMR tube and argon was blown over the sample which was then immediately tightly stoppered. The plot of % original CoA/hour that oxidized as a function of time is a straight line which is still linear at 118 hours. The forward rate of oxidation is 0.14% oxidation/hour, represented by the following reaction scheme:

$$2CoA \longrightarrow CoAS\text{-}SCoA + 2H^{+} + 2e^{-}$$

This value represents a minimum rate of oxidation for the previously purified and degassed samples. Buyske et al. (21) found that, at pH 10 and 25 °C, after 3 hours the CoA activity had decreased to 23.4% of its original activity. These results show the inadvisability of keeping CoA solutions exposed to the atmosphere for long periods of time at pH 10.

Most of the experiments were carried out on stock solutions prepared from CoA of 90% purity. The remaining 10% is due mainly to oxidized CoA as determined by NMR. However, for comparison purposes,

several experiments were carried out using stock solutions prepared from CoA of 100% purity. As shown in Table IIA, the relaxation time varies according to the supplier of CoA, but does not appear to be affected by the % of CoA in the oxidized state (i.e. sample 5 has ~ 16% of the CoA in the oxidized form whereas sample 6 has only 7%).

The best sample that was prepared had 7% of the CoA in the oxidized form (sample 6, Table II A) and this was obtained from the 100% stock solution only after electrolytic reduction of the CoA solution and careful degassing of the resin in a vacuum desiccator and consequent saturation with argon. Buffers and solvent were treated the same way. Samples 1 to 4 (from stock solutions prepared from 90% pure CoA) and sample 5 (from a stock solution prepared from 100% pure CoA) which was previously reduced had 10-15% of the CoA in the oxidized form. The argon treatment was not used for these latter samples.

The 100% pure CoA commercial preparation was investigated as follows: 10 milligrams of this material straight from the bottle were analyzed after each step. This CoA was dissolved in D_2O with a trace amount of EDTA and after storage at 4°C for 1 hour, its spectrum was taken. 16% oxidation was observed. According to Buyske et al.(21), the loss of activity of CoA after three hours should be 5% at pH 7 and T = 25°C. This sample was then electrolytically reduced and passed through the Chelex-100 column which was previously degassed and saturated with argon. Its spectrum showed 7% oxidation. The same result was obtained in the absence of EDTA (sample 6, Table IIA). The standard deviation of the average distance values for CoA-Mn(II) complex using

the CoA from the two sources are within 10%. This is a safely assumed uncertainty in r.

The above experimental results can be interpreted as follows : different preparations have a different response to storage conditions and column steps. For example, the 90% pure CoA stock solution seems to be more stable with an average degree of oxidation of only 12%. In order to minimize the oxidation products from the 100% CoA stock, more precautions are necessary ,including saturation of the buffers and resin with argon.

Calculation of the Correlation Time

The correlation time τ_c of the CoA-Mn(II) complex was evaluated from the water proton relaxation data of CoA-Mn(II) solutions at 100 MHz and 60 MHz (Table I).

Under the condition $(\omega_I \tau_c)^2 >> 1$, an exact value of τ_c and hence r is possible with a frequency dependent study. In the present study, $(\omega_I \tau_c)^2 < 1$. Different values of τ_c lead to different values of r, but the ratio of the distances will remain constant. τ_c was determined from the relaxation rates of the solvent water protons in the CoA-Mn(II) system and was used in the calculation of distances from the relaxation rates of the ^{31}P and ^{1}H nuclei. These calculations assume that the Mn-OH_2 (proton) distance of 2.87 Å observed for the haxaaquo Mn(II) complex (11) is unchanged in the CoA-Mn(II) binary complex and that the hyperfine contribution to $1/T_{1M}$ can be neglected , i.e. equation [10] is applicable.

TABLE I. Water proton relaxation times.

Sample[a]	Temp (°K)	Mn(II) (μ M)	T_1 Data		$T_{1M} \times 10^6$ (sec) [b]	
			$1/T_1$(Mn)	$1/T_1$(O)	$q=2H_2O$	$q=3H_2O$
I	287	114	4.00	0.455	1.16	1.74
II	287	114	4.42	0.503	1.05	1.58
III	298	86	3.03	0.385	1.17	1.76
IV	298	114	4.00	0.417	1.15	1.73

average τ_c [c] = $(6.6 \pm .06) \times 10^{-10}$ sec (q = 2)

average τ_c = $(4.0 \pm .1) \times 10^{-10}$ sec (q = 3)

	T_2 Data		T_{2p} (sec)
	$1/T_2$(Mn)	$1/T_2$(O)	
I	12.6	1.54	0.090
II	13.5	2.04	0.087
III	11.0	1.92	0.11
IV	13.5	1.26	0.082

(a) [CoA] = 61 - 62 mM in 0.05M Tris buffer containing 0.1M KCl, pH 8.2 .These samples were not degassed and their volume was 40 µ l. The fraction of free manganese ions is less than 2 %. 4K - 8K data points were used. Either the (180° - τ -90°) or the (180° - τ - - 90° - $5T_1$ - - 180° - τ - 90°)$_N$ sequence was used. Samples I, III and IV were done at 100 MHz and sample II, at 60 MHz.

(b) Calculated using equation [5].

(c) Determined from equation [10], using r = 2.87 Å .

That τ_c is applicable to both nuclei under consideration (i.e. water ^1H nuclei and CoA ^1H and ^{31}P nuclei) can only be true if the relaxation rates of both are dominated by the rotational correlation time. For the Mn(II) in the CoA-Mn(II) complex, τ_c is dominated by τ_R as shown below. For the hexaaquo Mn(II) complex, τ_m is 2.3×10^{-8} sec at 300°K (17), and hence for the complex, τ_m will also be much larger than τ_c. The τ_R was estimated from the tumbling time of the entire propionyl CoA molecule by the use of Stokes' law to be 6.2×10^{-10} sec (6). In addition, since the rotational correlation time is also much shorter than the electron relaxation time, T_{1e}, for Mn(II) in a nucleotide complex (10^{-8} to 10^{-9} sec) (14,25), τ_m^{-1} and T_{1e}^{-1} are neglected with respect to τ_R^{-1} in the analysis of the data for the CoA-Mn(II) complex. It is assumed that fast exchange of the water hydrogen is valid in this case. A significant contribution from τ_m for the water hydrogens will result in a shorter value of τ_c and consequently the various metal to nuclei distances in the complex calculated in this fashion will give an estimate for the minimum magnitude of r.

The value of $(\omega_s \tau_c)^2$ can now be calculated i.e. $(\omega_s \tau_c)^2 \gg 1$. In this case, it turns out that the term in equation [6] and [7] which is characterized by $(\omega_s \tau_c)^2$ can be neglected because its value is numerically insignificant relative to the term containing $(\omega_I \tau_c)^2$. In the Mn(II) complexes, $T_{2M}^{-1} \gg \Delta\omega_m$ (26). Moreover, since $T_{2M} \simeq T_{1M} \simeq 10^{-5} - 10^{-6}$ sec, $\tau_m^{-1} > T_{2M}^{-1}$, T_{1M}^{-1}, and it then follows that $(T_{2M} \tau_m)^{-1} > T_{2M}^{-2}$, $\Delta\omega_m^2$. This corresponds to fast exchange (equation [5]).

The average value of τ_c was obtained from four separate determinations of T_{1M} (Table I) using four separate samples where the Mn(II) ions were nearly totally bound to the CoA ($\frac{[\text{Metal free}]}{[\text{Metal total}]} \approx 2\%$). τ_c was calculated from equations [5] and [10] assuming that manganese coordinates to the CoA via four or three oxygen atoms, therefore two or three water molecules still coordinate to the Mn(II) ion (q = 2 or 3) in the complex. P_m is equal to Mn_b / 55.5 where Mn_b is the bound metal concentration. Under the present experimental conditions, the individual values of τ_c were found to be independent of the small change in temperature. The exact reason for this is not known. However one may speculate as to a possible reason. The temperature dependence of $\tau_c = \tau_R$ is given by equation [12] and predicts that as the temperature is decreased, the change in τ_R will be positive. Using equation [12] and a value of 4.9 Kcal $Mole^{-1}$ obtained from the NADP-Mn(II) system(27), then at 287 °K, τ_R is 40% longer than the corresponding value at 298°K. Such an effect can be compensated for by an increase in the number of water ligands in the CoA-Mn(II) complex at 287°K. In the NADP-Mn(II) (27) and the ADP-Mn(II) (28) systems, the Kd value decreases with temperature (10% increase for ADP for a temperature drop of 10°, i.e. a weaker complex at the lower temperature). If a weaker complex prevailed, one would expect the number of water ligands to increase somewhat. From Table I, we estimate that for 3 water ligands, the τ_R value is 40% lower than its corresponding value of 6.6×10^{-10} sec when q = 2, thus compensating for the above increase.

At 60 MHz , the shorter T_{1M} value (sample II, Table I) obtained can be accounted for by the $(\omega_I \tau_c)^2$ term of the dipolar equation [10].

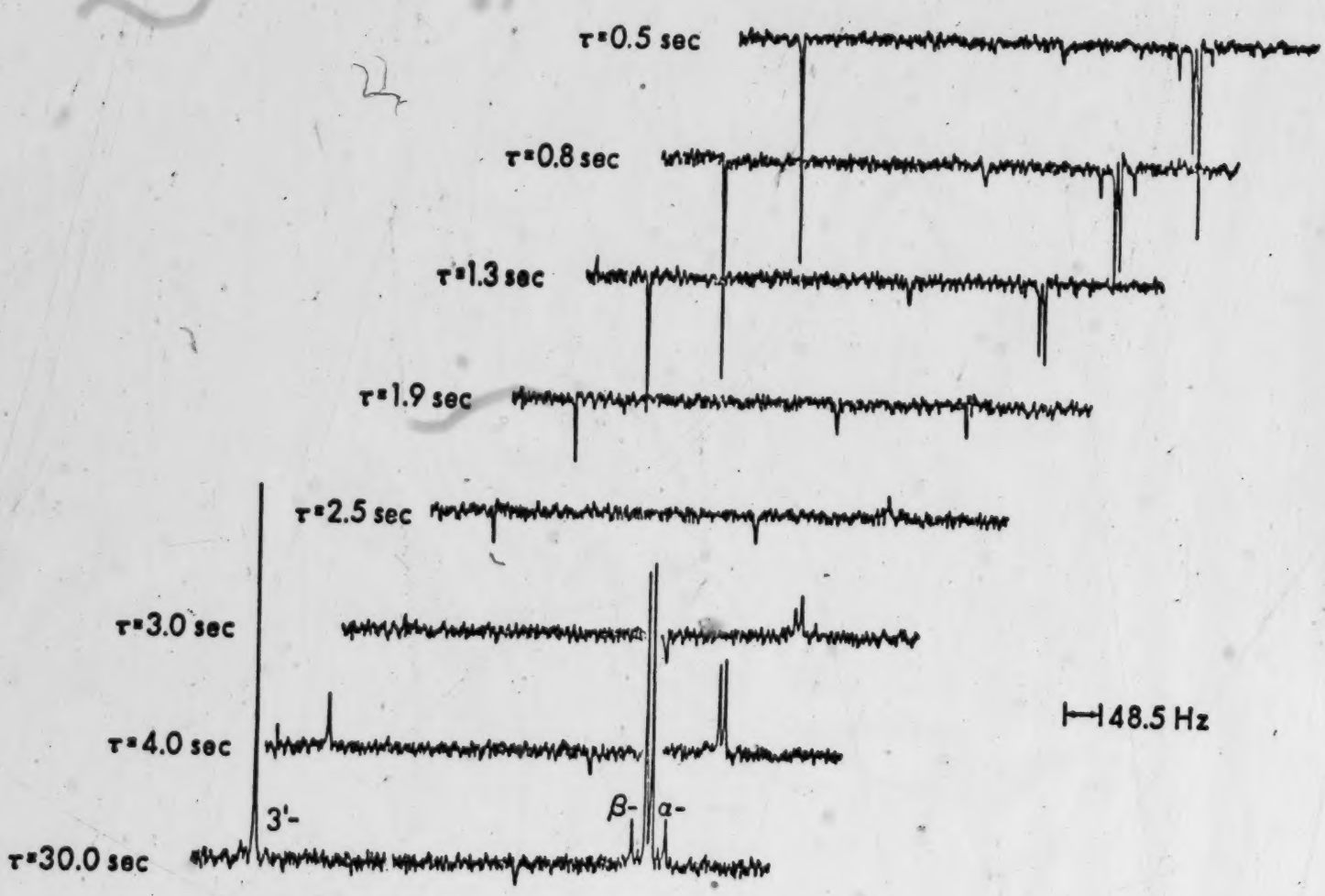
The average values of 4.0×10^{-10} or 6.6×10^{-10} sec for τ_c were used as the correlation time for the three phosphorus nuclei and for the various protons of CoA. Fung et al. (6) found that the correlation time of the three phosphorus atoms ($\tau_c = 2 \times 10^{-10}$ sec) of 0.010 M propionyl CoA were similar in magnitude to those of their nearest neighbor protons with correlation times of $1 - 3.5 \times 10^{-10}$ sec at 298°K. At 0.062M, τ_c, which is proportional to η (equation [13]) is expected to increase for the individual atoms because of the corresponding viscosity change and the possibility of self-association. ATP and dephospho CoA begin stacking at concentrations of 0.020 M (29) and >0.037 M (8) respectively. Since CoA has a greater charge (-4) than dephospho CoA (-2), it is most likely to stack at an even higher concentration.

In the above calculation of τ_c, the presence of two or three water molecules (q = 2 or 3) was assumed. The validity of these two values is supported by the observation that the new set of Mn(II) to phosphorus distances obtained using a lower concentration of CoA of 0.010 M at 298°K, and a τ_c of 2.0×10^{-10} sec, is in good agreement with the previous sets of distances obtained at a concentration of 0.062 M CoA and at 287° - 298° K(Table IV).

Phosphorus Magnetic Relaxation Time Measurements

Figure 2 shows a typical proton-decoupled ^{31}P NMR spectrum together with the inversion recovery spectra for the 3'-ribose-, β- and α -phosphorus nuclei of CoA. The relative position of the three phosphorus resonances is the same as previously described for propionyl CoA (6) and for CoA (1). The AB pattern arises because the magnitude of J and $\Delta\nu$

FIGURE 2. The proton decoupled inversion recovery ^{31}P NMR spectra obtained for CoA(0.010 M) at a pH of 7.7 in 0.05 M Tris buffer, 0.1 M KCl in D_2O. T = 298 °K. The sweep width is 800 Hz and each spectrum corresponds to 75 scans. The sample volume was 2.2 ml in a 10 mm NMR tube.


τ=0.5 sec
τ=0.8 sec
τ=1.3 sec
τ=1.9 sec
τ=2.5 sec
τ=3.0 sec
τ=4.0 sec
τ=30.0 sec
3'-
β-
α-
148.5 Hz

is similar. J is the spin coupling constant between the α- and β-phosphorus nuclei and $\Delta\nu$ is the chemical shift difference between them.

Table II summarizes five separate experiments representing six determinations of T_{1M} and T_{2p}. The blank line widths at half height (Table IIB) were on the average (0.84 ±0.05) Hz for the ribose 3'-phosphate and (1.1 ± ∿0.2 Hz) for the α- and β- phosphorus resonances respectively.

The ratio of the average $P_m T_{1p}$ and $P_m T_{2p}$ values for the three phosphorus nuclei is greater than or equal to six ($T_{1p}/T_{2p} \geq 6$). This result indicates that $T_{1M} > \tau_m$ and equation [5] can be used to calculate T_{1M} (9, 10, 23, 30). If $T_{2M} > \tau_m$, this will further imply that the scalar contribution to the transverse relaxation rate $(A/\hbar)^2 \tau_{el}$ in equation [7] dominates due to the delocalization of the unpaired spin density of the Mn(II) ion onto the phosphorus atoms and provides independent evidence for direct coordination of a ligand molecule to the Mn(II) site (14). The average T_{1M} values for the β-, α- and ribose $3'-^{31}P$ nuclei are equal within 10% (Table IIA).

Equation [10] was used to calculate the average r values using the two previously determined correlation times of 4.0×10^{-10} sec and 6.6×10^{-10} sec obtained from the relaxation rates of the water protons. The average values for T_{1M} in Table IIA correspond to the three phosphorus to Mn(II) ion distances of (3.5 - 3.7) Å and (3.8 - 4.0) Å for the two τ_c values of 4.0×10^{-10} sec and 6.6×10^{-10} sec, respectively. The average standard deviation computed from the large uncertainty in T_{1M} values and from an assumed uncertainty of 10 % in the expression for

TABLE II A. Paramagnetic effects of Manganese(II) ions on the longitudinal relaxation times of the CoA Phosphorus nuclei at 36.4 MHz and 287 °K.

Sample[a]	Solvent, pH	Phosphorus	$1/T_1(Mn)$ (sec^{-1})	$1/T_1(0)$ (sec^{-1})	T_{1p} (sec)	$T_{1M} \times 10^5$ (sec)
1.	D_2O	Ribose	1.18	0.294	1.13	3.17
	DTT[b]	β	1.15	0.388	1.31	3.68
	pH 7.7[c]	α	1.11	0.386	1.38	3.87
2.	H_2O	Ribose	1.24	0.435	1.24	3.54
	pH 6.3	β	1.22	0.385	1.20	3.42
		α	1.24	0.370	1.15	3.28
3.	D_2O	Ribose	1.16	0.333	1.21	3.45
	pH 6.5	β	1.45	0.417	0.968	2.76
		α	1.45	0.417	0.968	2.76
4.	H_2O	Ribose	1.30	0.417	1.13	3.18
	pH 7.6	β	1.32	0.400	1.09	3.05
		α	1.35	0.400	1.05	2.95

5.	H_2O	Ribose	0.599	0.248	2.85	8.02
	pH 7.8	β	0.719	0.270	2.23	6.27
		α	0.763	0.293	2.13	5.99
6.	H_2O(d)	Ribose	0.855	0.351	1.98	6.52
	pH 7.3	β	0.833	0.338	2.03	6.64
		α	0.847	0.356	2.04	6.69

Average $T_{1M} \times 10^5$ (sec) Ribose 4.6 ± 2.1

β 4.3 ± 1.7

α 4.3 ± 1.7

(a) 350 microliters of CoA ranging in concentration from 0.061 to 0.062 M were sealed in 5 mm NMR tubes. The Mn(II) concentration is 1.74×10^{-6}M for experiments 1 to 5 and 2.03×10^{-6}M for experiment 6. Samples 1 to 4 were prepared from the Na salt of CoA from Sigma Chemical Co.,and samples 5-6 were prepared from the Li salt from P.L. Biochemicals. Mn(II) free = 2%.

(b) [DTI] = 0.006 M.

(c) The buffer is 0.05M Tris containing 0.1M KCl for all solutions except 2 and 3 where 0.09M Tris in 0.1M KCl solution is used.

(d) CoA, straight from the commercial bottle, was electrolysed for 10 minutes at 6.43 milliamperes using the electrolysis cell of Saetre and Rabenstein (31). The resin and buffers were saturated with argon.

TABLE II B. Paramagnetic effects of Manganese(II) ions on the transverse relaxation times of the CoA Phosphorus nuclei at 36.4 MHz and 287 °K.

Sample[a]	Phosphorus	$1/T_2(Mn)$[b] (sec^{-1})	$1/T_2(0)$ (sec^{-1})	T_{2p}(sec)
1.	Ribose	8.36	2.67	0.18
	β	9.68	4.52	0.19
	α	9.46	3.20	0.16
2.	Ribose	11.3	9.42	0.53
	β	11.3	8.48	0.35
	α	12.6	9.42	0.31
3.	Ribose	12.3	2.58	0.10
	β	12.9	4.08	0.11
	α	14.8	3.77	0.091
4.	Ribose	15.4	2.51	0.078
	β	16.3	2.70	0.074
	α	15.7	3.27	0.080
5.	Ribose	4.90	2.54	0.42
	β	5.43	2.07	0.30
	α	5.43	2.89	0.39
6.	Ribose	9.74	2.89	0.15
	β	11.9	3.46	0.12
	α	11.9	3.46	0.12

(a) Sample compositions and conditions are given in Table II A.

(b) $1/T_2$ was calculated using equation [15].

τ_c is ≈8% (a sixth root is taken in equation [10]).

Two previous studies on the ATP-Mn(II) complexes at 0.003 M (23) and 0.10 M (29) indicate various degrees of dependence of r on the ATP concentration. At higher concentrations of ATP, formation of an aggregate of the $Mn(ATP)_N$ type is very likely since ATP stacks above 0.020 M. In view of this information, we have measured the Mn(II)-CoA system at a lower concentration (Table III) and used the correlation time measured for propionyl CoA of 2.0×10^{-10} sec previously determined by Fung et al. (6) who also worked at the same concentration and temperature i.e. 0.010 M and 298°K. These authors calculated the values of τ_R for several nuclei of the free propionyl CoA molecule by measuring the ratio of longitudinal relaxation rates at two frequencies (100 MHz and 220 MHz). If it is assumed that the value of the correlation time for the three phosphorus nuclei ($\sim 2 \times 10^{-10}$ sec) of propionyl CoA also apply to the phosphorus nuclei of the CoA-Mn(II) complex, then the determination of the distance, in this case, requires no information or assumption regarding the number of water molecules at the Mn(II) site since this is only necessary when the value of τ_R is determined by the water proton relaxation technique. The resulting distances of 3.5-3.7 Å (Table IVC)are in good agreement with the corresponding distances at 0.062 M CoA and at 287° - 298 °K. These results indicate that for the CoA-Mn(II) complex, a ^{31}P to Mn(II) distance of approximately 4 Å denotes a predominantly inner sphere metal complex (2.8 to 3.8 Å) (32). A second sphere complex for phosphorus requires a distance of 5.6 to 6.6 Å (32). The molecular structure of the phosphate is thus a tetrahedron where the

TABLE III. T_1 and T_2 data for the three Phosphorus nuclei at two CoA concentrations at 298 °K.

Sample	Phosphorus	T_1 Data		T_{1p}(sec)	$T_{1M} \times 10^5$(sec)
		$1/T_1$(Mn)	$1/T_1$(0)		
4.(a)					
pH 7.6	Ribose	1.24	0.385	1.17	3.28
[CoA] = 0.062 M in H_2O	β	1.08	0.333	1.34	3.76
[Mn(II)]= 1.74 $\times 10^{-6}$ M	α	1.25	0.357	1.12	3.14
7.(b)					
pH 7.7	Ribose	0.556	0.189	2.72	6.95
[CoA] = 0.010 M in D_2O	β	0.526	0.196	3.03	7.73
[Mn(II)] =0.283 x 10^{-6} M	α	0.526	0.233	3.41	8.67

		T_2 Data		T_{2p}(sec)	$(P_m T_{2p})^{-1} \times 10^{-4}$ (sec^{-1})
		$1/T_2$(Mn)	$1/T_2$(0)		
4.	Ribose	19.5	4.40	0.066	53.8
	β	15.7	4.40	0.088	40.3
	α	15.7	5.03	0.094	38.0
7.	Ribose	6.28	2.64	0.27	14.3
	β	6.03	2.51	0.28	13.8
	α	8.36	3.33	0.20	19.7

(a) Sample 4, Table IIA.

(b) This sample was sealed in a 10 mm NMR tube, and degassed three times. The T_{1M} values are corrected for the 10% uncomplexed Mn(II) ions. The buffer is 0.05 M Tris in 0.1M KCl solution ,T = 297°K. Sample 4 and 7 have the same [Mn(II)]/ [CoA] ratio.

TABLE IV. Values of the Mn(II)-Phosphorus nuclei distances for the CoA-Mn(II) complex.

Sample	[CoA](M)	T (°K)	r(Å)		
			3'-ribose	β	α
A. $q = 2H_2O$, $\tau_c = 6.6 \times 10^{-10}$ sec					
See Table IIA, 1-6	0.062	287(6)[a]	4.0 ±.4[b]	4.0 ± .4	4.0 ± .4
See Table III,4	0.062	298(1)	3.8 ±.4	3.9 ± .4	3.8 ± .4
B. $q = 3H_2O$, $\tau_c = 4.0 \times 10^{-10}$ sec					
See Table IIA, 1-6	0.062	287(6)	3.7 ±.4	3.7 ± .4	3.7 ± .4
See Table III,4	0.062	298(1)	3.5 ±.4	3.6 ± .4	3.5 ± .4
C. $\tau_c = 2.0 \times 10^{-10}$ sec[c]					
See Table III,7	0.010	298(1)	3.5 ±.4	3.6 ± .4	3.7 ± .4
D. Corey-Pauling-Koltun Model[d]			3.6	3.8	3.8

(a) Refers to the number of T_{1M} determinations.

(b) According to Mildvan (32), the lowest error limits in r are ±10%. However, the error computed from random errors of the component parameters τ_c and T_{1M} by squaring and adding the individual relative standard deviations and dividing the final result by six (a sixth root is taken in equation [10]) was less than this value.

(c) This correlation time is for the phosphorus nuclei for 0.010 M propionyl CoA as determined by Fung et al. (6)

(d) The uncertainty in measuring the distances of the CPK model is estimated to be± 0.3 Å.

manganese to the phosphorus distance of ~ 4 Å is consistent with a simple model where the ion is chelated by an oxygen from each of the phosphates of the pyrophosphate group and by one or two oxygens from the 3'-phosphate, resulting in an inner sphere metal complex.

The value of 3.5 - 4.0 Å could also be the result of a time or space average of 45% inner sphere (3.3 Å) and 55% second sphere complex -es (6.1 Å) in a rapid equilibrium with a rate faster than the observed relaxation rate T_{1M}^{-1} ($> 10^5$ sec^{-1}) (34). This possibility has been explored for the non complexed pyrophosphate moiety by Lee et al. (5) who found that in free solution, the pantetheine tail undergoes total freedom in segmental motion via rotation about the P-O-P bond.Applying the above calculation to the 3'-phosphate will result in a space average for the sugar moiety of the CoA-Mn(II) complex of 45% and 55% in the ^3E and ^2E state respectively.

Proton Magnetic Relaxation Time Measurements

The assignment of the proton resonances of CoA and of a number of CoA derivatives has been previously described (3, 5, 6, 35). Figure 1 shows the structure of CoA with the adenine base in the anti conformation. The Fourier transform proton magnetic resonance spectrum of CoA is shown in Figure 3 and the inversion recovery spectra of the AH(8), AH(2) and AH(1') protons are shown in Figure 4. In the presence of various Mn(II) ion concentrations , the line width of AH(8) (Table V) undergoes conspicuous broadening whereas the AH(2) proton was only slightly affected. In agreement with the criteria of Chan and Nelson, this suggests a preferential anti orientation as opposed to a syn conformation (36). Lee and Sarma (5) have clearly shown by chemical shift measurements that

FIGURE 3. The Fourier transform, proton magnetic resonance spectrum of CoA (0.061 M) at 100 MHz in D_2O solution at 298 °K. Measurements were made in 0.050 M Tris containing 0.1 M KCl, pH 8.1, with a final volume of 350 microliters. Eight free induction decays were collected. The HDO peak was saturated.

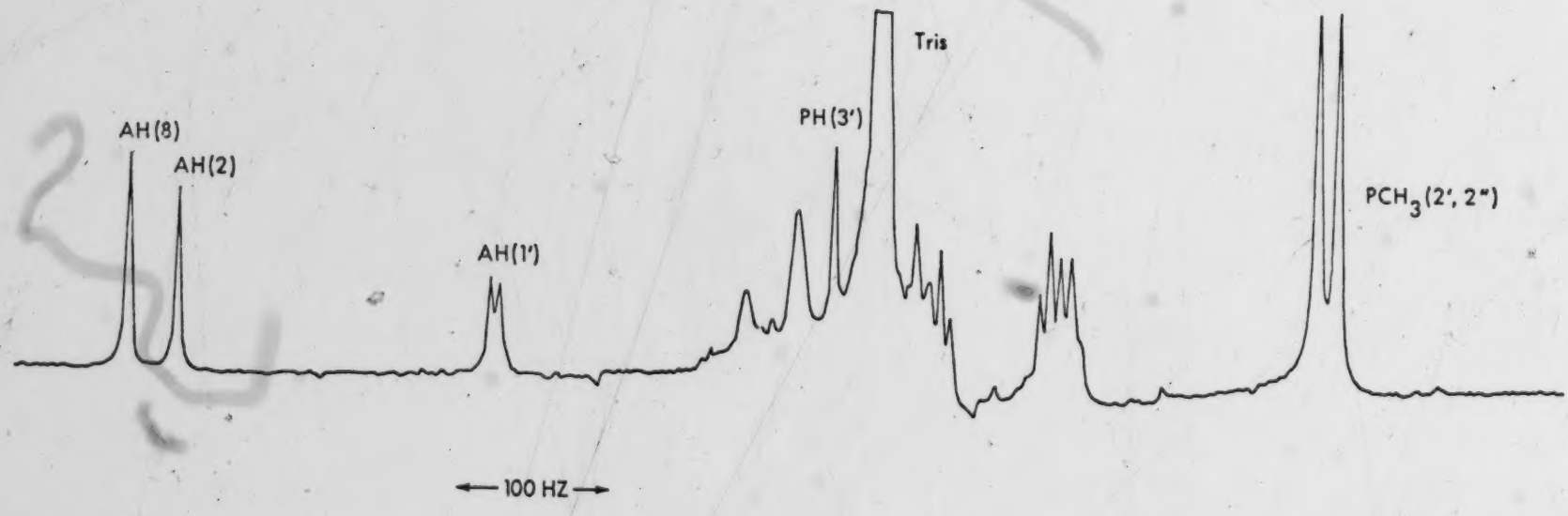
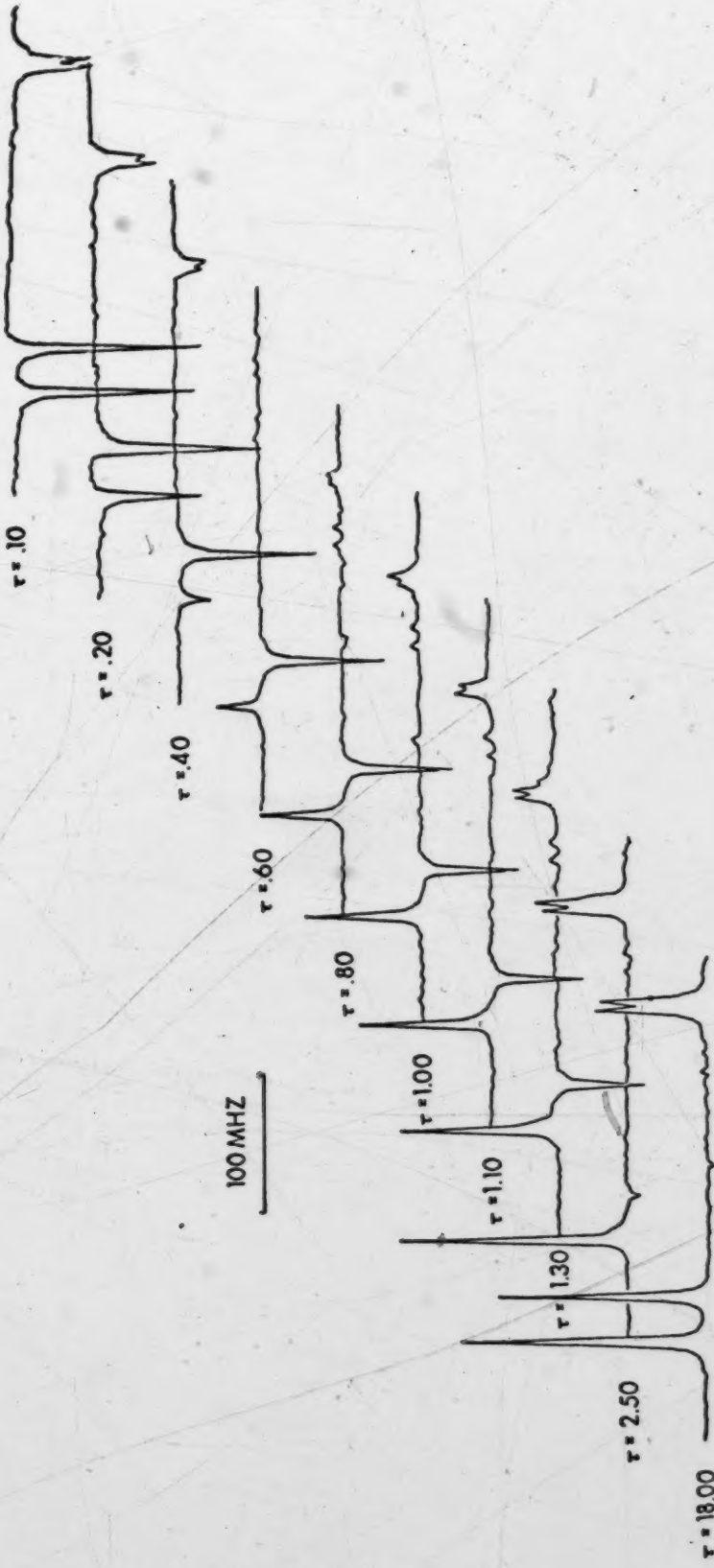

AH(8)
AH(2)
AH(1')
PH(3')
Tris
PCH3(2', 2")
100 HZ

FIGURE 4. The inversion recovery NMR spectra for the AH(8), AH(2), and the AH(1') protons of CoA in the absence of manganese ions. Experimental conditions were as described for Figure 3.


τ = .10
τ = .20
τ = .40
τ = .60
τ = .80
τ = 1.00
τ = 1.10
τ = 1.30
τ = 2.50
τ = 18.00
100 MHZ

in the absence of bound Mn(II), the AH(8) shift is highly sensitive to ionization of the pyrophosphate portion and consequently, as well concluded that the adenine base is in the preferred anti orientation. Sloan et al. (23) found, from a molecular model of the ATP-Mn(II) complex constructed from six calculated metal to nucleus distances, that the adenine base is also in agreement with the above conformation.

The longitudinal relaxation rates of the CoA protons at 60 MHz and 100 MHz are summarized in Tables V and VI. The metal ion increased the relaxation rate of the AH(8) proton of the adenine ring to the greatest extent while influencing the T_{1p}^{-1} of AH(2), AH(1') and PH(3') to a lesser extent. The methyl proton T_1 values of the pantetheine moiety were not affected by the Mn(II) ions. As a result of the strong HDO resonance ($\sim$ 1M) and of the complex spectrum observed for the various other protons, the measurement of the relaxation rates of the other CoA protons are impossible with the presently available field strength and these solution conditions.

The effect of manganese (II) ions on the proton longitudinal relaxation rates is shown in the plot of [CoA] $\left(\frac{1}{T_1(Mn)} - \frac{1}{T_1(0)} \right)$ versus [Mn(II)] (Figure 5).

The above normalized proton relaxation rates (Table V and VI) can be used to calculate the various metal to proton distances providing that they are not exchange-limited. None of the calculated proton longitudinal relaxation rates (Tables V and VI) approximate the normalized transverse relaxation rate of 5.4×10^5 sec $^{-1}$ at 298 $^{\circ}$K observed for the

TABLE V. Paramagnetic effects of Manganese (II) on longitudinal relaxation rates of the CoA protons at 100 MHz.

Sample[a]	Proton[b]	$1/T_1$(Mn) (sec^{-1})	$1/T_1$(0) (sec^{-1})	T_{1p} (sec)	$T_{1M} \times 10^5$ (sec)
1. pH 8.4	AH(8)	8.33	1.56	0.148	2.81
[Mn(II)]=11.6x10^{-6}M	AH(2)	1.19	0.263	1.08	20.5
	AH(1')	2.33	0.909	0.704	13.4
	PH(3')	3.33	1.45	0.532	10.1
2.[c] pH 7.7	AH(8)	6.25	1.39	0.206	2.27
[Mn(II)]=6.94x10^{-6}M	AH(2)	1.08	0.303	1.29	14.3
	AH(1')	1.85	0.758	1.09	12.0
	PH(3')	2.27	1.12	0.870	9.59
3. pH 7.7	AH(8)	5.56	1.39	0.240	1.76
[Mn(II)]=4.63x10^{-6}M	AH(2)	0.769	0.303	2.15	15.8
	AH(1')	1.15	0.758	2.55	18.7
	PH(3')	2.00	1.12	1.14	8.39
4.[d] pH 8.1	AH(8)	6.76	1.29	0.183	2.77
[Mn(II]=9.26x10^{-6}M	AH(2)	1.15	0.228	1.08	16.3
	AH(1')	1.83	0.848	1.02	15.4
	PH(3')	2.48	1.14	0.746	11.3

5. pH 8.1	AH(8)	10.3	1.29	0.111	2.52
$[Mn(II)]=13.9\times10^{-6}M$	AH(2)	1.73	0.228	0.666	15.1
	AH(1')	2.65	0.848	0.555	12.6
	PH(3')	3.45	1.14	0.433	9.83
6. pH 8.1	AH(8)	15.7	1.29	0.0694	2.10
$[Mn(II)]=18.5\times10^{-6}M$	AH(2)	2.38	0.228	0.465	14.1
	AH(1')	3.79	0.848	0.340	10.3
	PH(3')	4.15	1.14	0.332	10.0

Average $T_{1M} \times 10^5$(sec)

AH(8)	2.43 ± 0.43[e]
AH(2)	16.0 ± 2.4
AH(1')	13.7 ± 3.0
PH(3')	9.87 ± 0.94

(a) The relaxation rates were measured on the Varian HA-100 NMR spectrometer, T= 298°K, [CoA] = 0.062 ± 0.001 M, in 0.05M Tris, 0.1 M KCl buffer in D_2O. [Mn(II)] free≈ 2%.

(b) The average $(P_m T_{2p})^{-1} \times 10^{-4}$ values as determined from line width measurements for AH(8), AH(2), AH(1') and PH(3') resonances are 6.7 ± 1.3, 1.3 ± 1.0, 2.0 ± 0.9 and 0.54 ± 0.46 sec^{-1}, respectively. These large uncertainties are due to the magnetic field inhomogeneity. These values were not used in any of the distance calculations.

(c) This sample was not sealed.

(d) Water suppression was used for samples 4, 5 and 6.

(e) The experimental relative standard deviation for $T_{1M} \times 10^5$ values are 18 %, 15%, 22% and 10% for the above protons respectively.

TABLE VI. Paramagnetic effects on the longitudinal relaxation times of the CoA protons at 60 MHz.

Sample[a]	Proton	$1/T_1(Mn)$ (sec^{-1})	$1/T_1(0)$ (sec^{-1})	T_{1p} (sec)	$T_{1M} \times 10^5$ (sec)
7. DTT[b]	AH(8)	3.72	2.25	0.680	1.91
pH 7.7	AH(2)	0.658	0.422	4.24	11.9
$[Mn(II)] = 1.74 \times 10^{-6}$ M	AH(1')	1.80	1.41	2.56	7.20
	PH(3')	2.26	2.07	5.26	14.8
8. DTT[c]	AH(8)	5.24	2.25	0.334	2.50
pH 7.7	AH(2)	1.20	0.422	1.29	9.60
$[Mn(II)] = 4.63 \times 10^{-6}$ M	AH(1')	3.08	1.41	0.599	4.47
9. pH 8.2	AH(8)	7.14	2.51	0.216	1.74
$[Mn(II)] = 5.00 \times 10^{-6}$ M	AH(2)	1.58	0.433	0.872	7.03
	AH(1')	3.00	1.50	0.667	5.38
	PH(3')	3.33	2.07	0.794	6.42

Average $T_{1M} \times 10^5$ (sec)

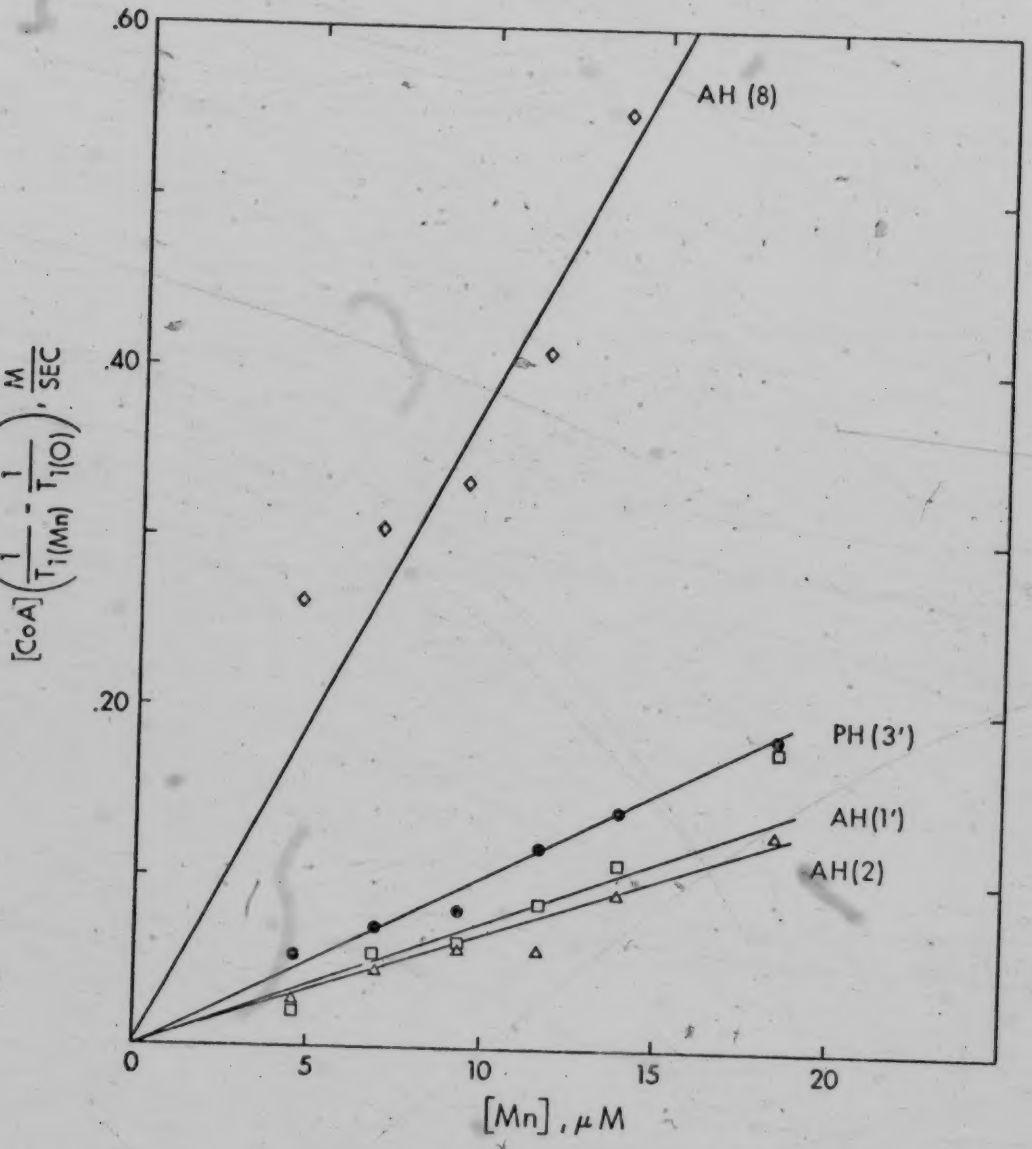
AH(8)	2.05± 0.40(20%)
AH(2)	9.51± 2.40(25%)
AH(1')	5.68± 1.4 (25%)
PH(3')	10.6 ± 5.9 (56%)

(a) T = 286.5 ± 0.5 °K, [CoA] = 0.062 M in 0.05M Tris, 0.1M KCl buffer, in D_2O. The line broadening was generally insensitive to these low manganese concentration, except for the AH(8) resonance.

(b) Same as sample 1 in Table II A.

(c) [DTT] = 0.006 M. The PH(3') baseline is affected by the adjacent strong Tris resonance.

FIGURE 5. The effects of manganese ions on the longitudinal relaxation rates (Table V) of the four protons of CoA at 100 MHz. Experimental conditions were as described in Table V.


.60
.40
.20
0
5
10
15
20
[Mn], μM
$[CoA]\left(\frac{1}{T_{1(Mn)}} - \frac{1}{T_{1(O)}}\right)$, $\frac{M}{SEC}$
AH (8)
PH (3')
AH(1')
AH(2)

β-phosphorus atom (Table III, sample 4). Since chemical exchange refers to both nuclei as they are on the same molecule, this value of $(P_m T_{2p})^{-1}$ allowed the determination of an upper limit of 1.9×10^{-6} sec for the exchange lifetime, τ_m (298°K) of CoA on the metal ion. All the longitudinal relaxation rates listed in Table V are at least 9.5 times greater and are therefore not exchange-limited by this criterion (23, 37) i.e. $(T_{1M} > \tau_m)$.

The absence of any effects due to the CoA concentration and the validity of our values must be supported by another set of data at lower concentration. In order to determine concentration effects, the proton resonance shifts of the adenine moiety of CoA(0.042 M)at two temperatures were obtained to see whether molecular association was taking place. The spectra show that the lowest field, AH(8) peak is not affected by temperature. However the AH(2) resonance was shifted to lower field by ∿ 4Hz with a change in temperature from 5 °C to 30 °C. Shifts of this nature can be attributed to magnetic anisotropy associated with ring current effects in neighboring molecules. A weak molecular association via stacking of the adenine rings will result in a similar shift to lower field ([CoA]> 0.037 M). A horizontal interaction via hydrogen bonding would produce shifts in the opposite direction. In order to explain the AH(8) chemical shift insensitivity to temperature, the ring axes must be somewhat displaced. Chan et al. in their NMR study of the self-association of purine (38), have shown graphically that at∿ 0.050 M purine, the AH(8) and AH(2) chemical shifts are 2 to 4 Hz to lower field at 50 °C than 25 °C. Lacking a detailed study of CoA chemical shift versus

concentration, which is needed to explain the origin of the observed small shift of the CoA proton, we proceeded to measure the proton relaxation rates of a 0.010 M CoA solution to verify the distances calculated for 0.062M CoA solutions. The calculated metal to proton distance of the 0.010 M CoA solution are in good agreement with the previous results at 0.062 M. One such experiment is shown in Table VII and Table VIII summarizes the distances between the Mn(II) ion and the four proton nuclei of CoA which were calculated using the Solomon-Bloembergen equation [10]. The results obtained at 100 MHz are within 10% of the distances similarly determined at 60MHz (Table VI).

The overall proton distances fit a model where the metal ion is bound above the sugar ring. Furthermore, the distances between the A(5'-5") proton and the A(4') proton and the Mn(II) site, estimated from the null point (equation [17]) in the inversion recovery experiment,are $\sim$ 4.4 Å and $\sim$4.8 Å respectively and are consistent with the above conclusion. Only an approximate calculation of the null point value was made because of the interference from the HDO and Tris resonances.

The position of the CoA protons relative to the metal ion are approximately the same as those for the ATP-Mn(II) complex (23) where AH(8) = 4.5 Å, AH(2) = 6.4 Å and AH(1') = 6.2 Å. The resulting model shows that the ATP folds around the metal ion above the sugar plane. A 3'-endoribose ring puckering (^3E) and a gauche-gauche conformation for the C(4')-C(5') bond were assumed in the ATP-Mn(II) model. The concentration was $\sim$ 0.003 M and a $\tau_c = 1.0 \times 10^{-10}$ sec was used. In constrast, a 0.35 M ATP solution (39) with $(0.1 - 1) \times 10^{-3}$ M Mn(II) ion concentration has different corresponding values of the distances, namely 4.5 Å, 5.7 Å and

TABLE VII. T_1 data for the Proton nuclei at a CoA concentration of 0.010 M at 100 MHz.

Sample	Proton	$1/T_1(Mn)$ (sec^{-1})	$1/T_1(0)$ (sec^{-1})	T_{1p}(sec)	$T_{1M} \times 10^5$ (sec)
12.[(a)] pH 6.9	AH(8)	4.10	1.16	0.340	5.85
[CoA]=0.010 M in D_2O	AH(2)	0.562	0.192	2.70	46.5
[Mn(II)]= 1.98 x 10^{-6} M	AH(1')	1.39	0.685	1.42	24.4

(a) The $(P_m T_{2p})^{-1} \times 10^{-4}$ values for AH(8), AH(2) and AH(1') resonances are 9.2, 6.7 and 6.8 sec^{-1} respectively. T_{1M} values are corrected for the 10% uncomplexed Mn(II) ions.

Table VIII. Values of the Mn(II)-Proton nuclei distances.

Sample	[CoA] (M)	T (°K)	r (Å)			
			AH(8)	AH(2)	AH(1')	PH(3')
A. $q = 2H_2O$, $\tau_c = 6.6 \times 10^{-10}$ sec						
See Table V, 1 - 6	0.062	298(6)[a]	4.8 ±.5	6.5 ±.6	6.4 ±.6	6.0 ±.6
see Table VI,7 - 9	0.062	287(3)	4.7 ±.5	6.1 ±.6	5.6 ±.6	6.0 ±.6
(Not shown),10 -11	0.062	298(2)	4.7 ±.5	6.1 ±.6	—	—
B. $q = 3H_2O$, $\tau_c = 4.0 \times 10^{-10}$ sec						
See Table V, 1 - 6	0.062	298(6)	4.5 ±.5	6.1 ±.6	5.9 ±.6	5.6 ±.6
See Table VI,7 - 9	0.062	287(3)	4.4 ±.4	5.6 ±.6	5.2 ±.5	5.7 ±.6
(Not shown),10 -11	0.062	298(2)[b]	4.4 ±.4	5.6 ±.6	—	—
C. $\tau_c = 2.0 \times 10^{-10}$ sec [c]						
See Table VII, 12	0.010	298(1)	4.6 ±.5	6.5 ±.6	5.9 ±.6	—
D. Corey-Pauling-Koltun Model			4.8	7.8	6.0	6.0

(a) Number of T_{1M} determinations.

(b) One blank and two metal solutions were used for two determinations of T_{1M}. Measurements (not shown) were made at 60 MHz at 298 °K in 0.09 M Tris buffer in 0.1 M KCl solution, pH 6.5. The CoA (0.062 M) protons relaxation times were measured in the presence of 1.74 and 4.63 x 10^{-6} M manganese (II) chloride. The average $(P_m T_{2p})^{-1} \times 10^{-4}$ values for AH(8) and AH(2) resonances for the 1.74 and 4.63 x 10^{-6} M Mn(II) solutions are 8.5± 4.2 and 3.7 ±1.5 sec^{-1}, respectively.

(c) This correlation time is for 0.010 M propionyl CoA as determined by Fung et al. (6)

7.4 Å and the τ_c value used was 10×10^{-10} sec. Fazakerley et al. (8) found that the distances between the AH(8), AH(2), AH(1') and PH(3') protons and the lanthanide ions in the lanthanide ion complexes with dephospho CoA were all $\geq$ 8.0 Å and that the pyrophosphate part of the chain is folded backward whereas the remainder of the chain is extended downward. From the lanthanide ion -dephospho CoA model, the ratio r(AH(i))/ r(AH(1')) is 0.94 , 1.03 and 0.93 for protons AH(8), AH(2) and PH(3') respectively. Similarly, this ratio for the present CoA results (Table VIII) is 0.75, 1.01 and 0.94 respectively .

CHAPTER 5

MOLECULAR MODEL FOR THE CoA-Mn(II) COMPLEX

In the calculation of the distances of the various nuclei from the metal ion centre, it was assumed that the polydentate ligand CoA still forms predominantly a 1:1 complex of high stability at 0.062M and therefore the problem of a series of complexes of CoA overlapping each other was neglected.

The seven calculated metal to nucleus distances were used to build a 3-dimensional structure for the CoA-Mn(II) complex using Corey-Pauling-Koltun (CPK) precision molecular models. In constructing the folded model, we have attempted to generate a geometry which can be explained by consideration of the various distances and observations previously mentioned.

In free solution, the folded and linear conformations of free CoA are known to adopt the same preferred conformations except for two major differences. These involve the rotation about the P-O-P and the N-C(5') bonds (5). In constructing the model, those conformations for which there is an overwhelming preference in free solution (5) were used where possible. Therefore, in designing the molecular model for the complex, the following criteria were used:

1. A 3'-endoribose ring puckering (^{3}E) where the electrostatic repulsive interaction between the 3'- and 5'- phosphate groups is minimized by the positive charge of the manganese. In free solutions(5), the molecule assumes mainly the ^{2}E pucker conformation

(81% $^{2}E \rightleftharpoons$ 19% ^{3}E, at pH 8.0). At pH 5.0, 78% of the sugar moieties assume the ^{2}E conformation whereas at pH 1.5 this value drops to 68%. Dephospho CoA (8) at pH 8.0 has an ^{3}E population which is 11% higher than that of CoASH at the same pH due to the removal of the electrostatic repulsion between the 3'- and 5'- phosphate (5). This latter conformation positions the 3'-phosphate atom slightly above the plane of the sugar ring (Figure 6a);

2. A gauche-gauche conformation for the exocyclic linkage of the adenosine part (Figure 6b:1) (5);

3. A staggered conformation for the 3'-phosphate(Figure 6b: 14) (5). This conformation brings the phosphorus atom closer to the pyrophosphate moiety;

4. A dihedral angle between AH(8) and AH(1') (as defined by Schirmer et al. (40) of ~135°, i.e. predominantly an anti conformation derived from the adenine proton r values;

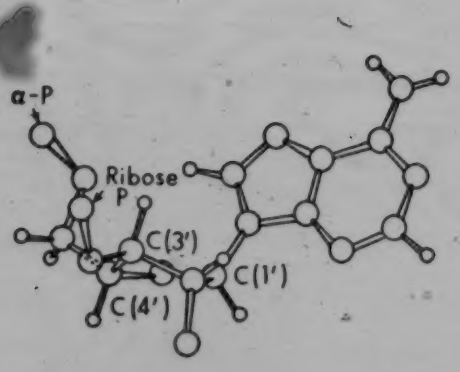
5. The conformation about the various pantetheine atoms PC(1')-PC(2'), PC(2')-PC(3') and PC(3')-PC(4') (Figure 6b: 5 to 7) were left in the maximum staggered orientation (5). Considering the large number of bonds about which rotation can occur, it is unlikely that the pantetheine pyrophosphate fragment of CoA will adopt a unique conformation in solution.

6. A metal-to-oxygen distance of 2.3 Å was used and two oxygen atoms from the pyrophosphate group and one from the ribose 3'-phosphate were assumed in the construction of the CoA-Mn(II) model. In $Co(NH_3)_4H_2P_3O_{10}$

FIGURE 6. a. A possible structure of the sugar conformation.

b. Newman projections about the various bonds of the complex. Some of these drawings were taken from Ref. 1.

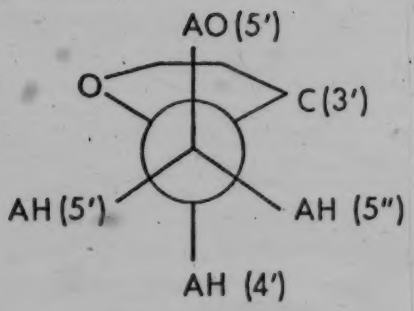
a)



Anti - ^{3}E - gg - conformation

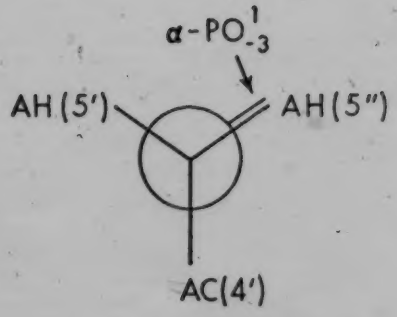
b)

1



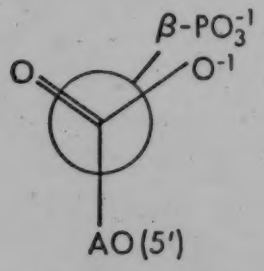
GAUCHE - GAUCHE EXOCYCLE LINKAGE AC(4') - AC(5')

2



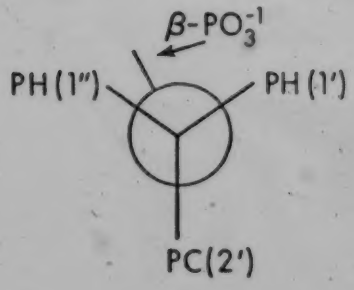
AC(5') - AO(5') Bond

3



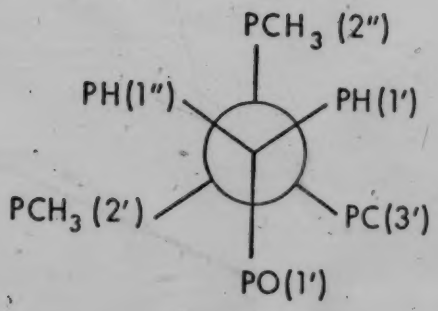
α-PHOSPHORUS-O-β-PHOSPHORUS LINKAGE

4



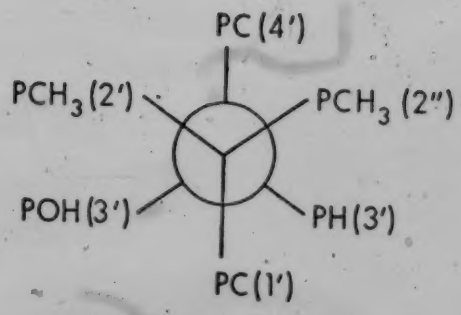
PC(1') - PO(1') Bond

5



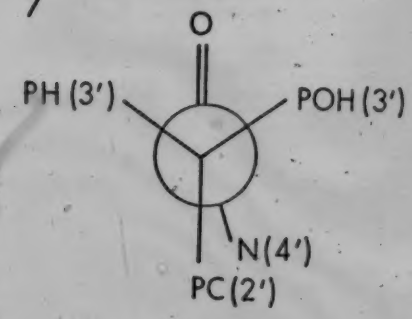
PC(1') - PC(2') Bond

6



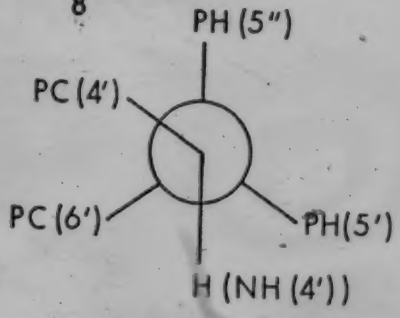
PC(2') - PC(3') Bond

7



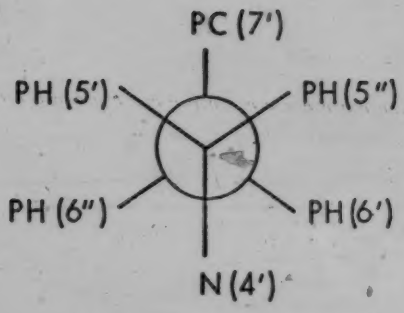
PC(3') - PC(4') Bond

8



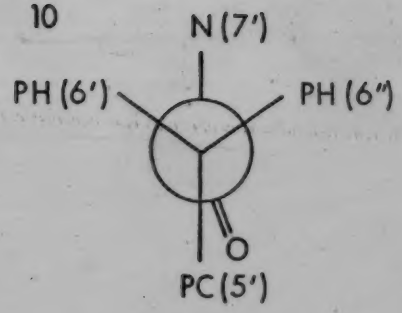
PN(4') - PC(5') Bond

9



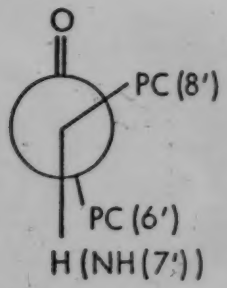
PC (5') - PC (6') Bond

10



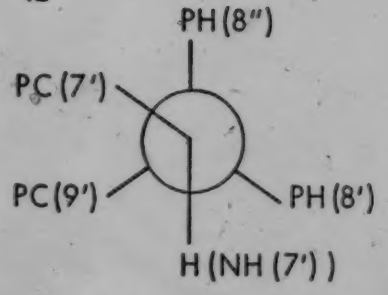
PC (6') - PC (7') Bond

11



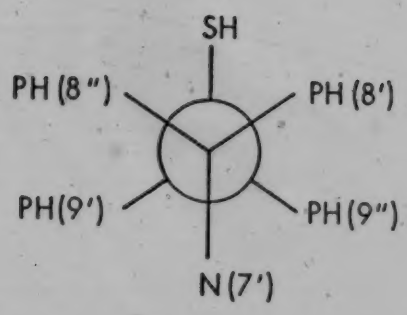
PN (7') - PC (7') Bond

12



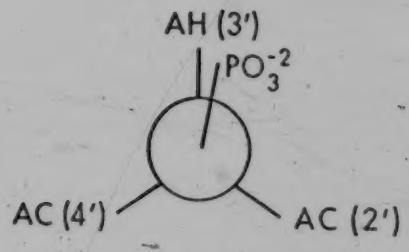
PN (7') - PC (8') Bond

13



PC (8') - PC (9') Bond

14



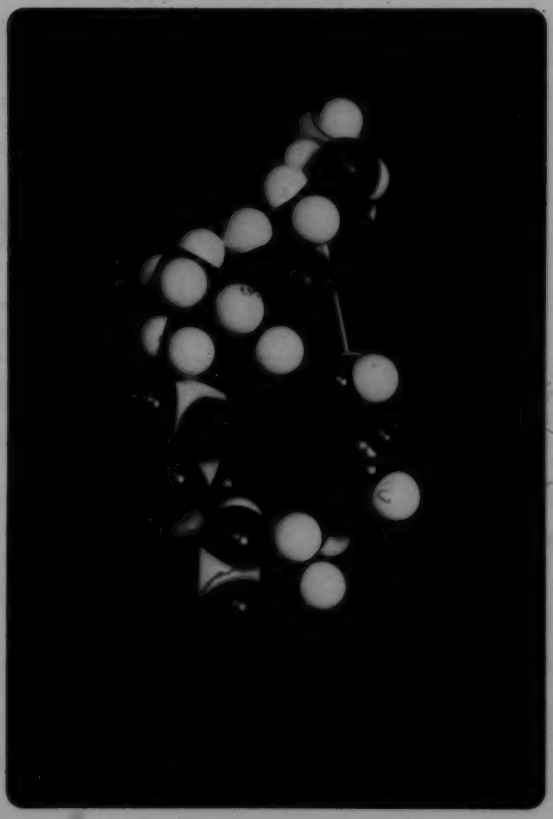
3'- Phosphate Oxygen
- AC (3') Linkage

crystal, the bond distance between the metal ion and the oxygen atom is 1.94-1.95 Å (41). For the lanthanide complex of dephospho CoA, Fazakerley et al. (8) found that the lanthanide oxygen distance of 2.3 Å best fits their data.

One possible model for the conformation of the CoA-Mn(II) complex that is consistent with the present data and with the above criteria is shown in Plate 1. Various Newman projections illustrate the framework of the complex (Figure 6b: 1 - 14). The distances between the Mn(II) ion (not shown in Plate 1) and the three phosphorus nuclei and the four proton nuclei in the CPK model are given at the bottom of Tables IV and VIII respectively. The measured distances from the Mn(II) ion to the AH(2) proton of (5.6 - 6.5)Å for the two values of τ_c (4.0×10^{-10} sec and 6.6×10^{-10} sec) (Table VIII), in comparison with the distance observed from the CPK model (7.8 Å) , are not completely consistent with the adenine conformation of ~135° about its glycosidic bond. Some contribution to the distances from a syn conformation is likely to provide an adequate explanation for such a discrepancy since the motion of the adenine ring about its glycosidic bond will make long distances appear shorter through the time averaging scheme (34). Fazakerley et al. (8) found that an excellent fit of the relaxation data arises when the dephospho CoA molecule spends 7% of the time in the syn form and 93% of the time in the anti form.

An attempt to make a model of CoA-Mn(II) similar to the model of the dephospho CoA-lanthanide complex constructed by Fazakerley et al. (8) failed to produce an acceptable conformation corresponding to the various r values.

PLATE 1. A possible Corey-Pauling-Koltun molecular model for the conformation of the CoA-Mn(II) complex.



In agreement with the value of 2 or 3 water molecules (q = 2 or 3) and with the various distances, it can be seen that direct coordination of the metal ion to the CoA is via three or four phosphoryl ligand oxygens. Two oxygen atoms from the pyrophosphate group and one or two from the ribose phosphate are involved. The 3'-phosphate to Mn(II) distance of (3.5 - 3.7) Å (Table IV B) is in good agreement with the distance of 3.3 Å obtained between the manganese ion and the phosphorus nucleus in the $LiMnPO_4$ crystal (42).

Summary

A crystallographic determination of the structure of CoA has not been done, probably because of the difficulty in obtaining pure crystals. Our present results show that the best-fit structure is L-shaped about the Mn(II) ion. The metal is coordinated to three or four oxygen donors of the pyrophosphate linkage and the 3'-ribose phosphate. The ribose is in the ^3E conformation with the adenine base predominantly in the anti conformation. The chain is extended upward. The local conformation of the individual portions of CoA beyond PC(3')(i.e. pantetheine moiety)are not yet defined. More distances are needed to complete the conformation of CoA. Finally ,the exact number of bound water molecules in the CoA-Mn(II) complex cannot be determined directly from our data and the time averaged value need not be an interger. Nevertheless, in the present study, this number has been estimated to be between 2 and 3 based on measurements on 0.010 M CoA and using a correlation time from a 0.010 M propionyl CoA solution (6).

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34. The % time spend in the inner and second sphere complex, x and y respectively, is calculated from the following equations : $x + y = 1.0$, $x \left(\frac{1}{r_1}\right)^6 + y \left(\frac{1}{r_2}\right)^6 = \left(\frac{1}{r_{obs}}\right)^6$, where r_{obs} is the observed value and r_1 and r_2 are the values of the distance in a given conformation. This scheme value is averaging the distance raised to the minus six power rather than the distance raised to the power one (33).

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